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ORIGINAL ARTICLE

Ventral Pallidum Encodes Contextual Information and Controls Aversive Behaviors

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Abstract

Successful avoidance of aversive outcomes is crucial for the survival of animals. Although accumulating evidence indicates that an indirect pathway in the basal ganglia is involved in aversive behavior, the ventral pallidum (VP), which is an important component of this pathway, has so far been implicated primarily in appetitive behavior. In this study, we used single-cell recordings and bicuculline ($GABA_A$ antagonist) injections to elucidate the role of VP both in the encoding of aversive context and in active avoidance. We found 2 populations of neurons that were preferentially activated by appetitive and aversive conditioned stimuli (CSs). In addition, VP showed appetitive and aversive outcome anticipatory activities. These activity patterns indicate that VP is involved in encoding and maintaining CS-induced aversive contextual information. Furthermore, the disturbance of VP activity by bicuculline injection increased the number of error trials in aversive trials. In particular, the subjects released the response bar prematurely, showed no response at all, or failed to avoid the aversive outcome. Overall, these results suggest that VP plays a central role in controlling CS-induced negative motivation to produce avoidance behavior.

Key words: active avoidance, anxious-like behavior, aversive, nonhuman primate, ventral pallidum

Introduction

The loss of regulation in aversive behavior results in inappropriate behavioral responses. For instance, patients with anxiety-related disorders, such as post-traumatic stress disorder (PTSD) and obsessive–compulsive disorder (OCD), show excessive avoidance behaviors and loss of adaptive anxiety regulation (Stein and Paulus 2009; Grupe and Nitschke 2013; Gillan et al. 2014). In contrast, if animals do not take an appropriate action in aversive context, harmful results, such as facing fear, injury, and death, could occur. Thus, adequate processing of an aversive event is crucially important for survival (LeDoux J 2012; LeDoux JE 2012). In a typical case, the avoidance of aversive events can depend on the anticipatory aversive events, which arise from learned associations between a conditioned stimulus (CS) and a subsequent aversive unconditioned stimulus (US).

The basal ganglia consist of a direct and indirect pathway (Albin et al. 1989; DeLong 1990), which appear to play complementary roles in action selection. While the direct pathway seems to be primarily involved in appetitive approach behavior, the indirect pathway may be more involved both in the avoidance of aversive behaviors and in the inhibition of competing actions (Bateup et al. 2010; Hikida et al. 2010; Kravitz et al. 2012). The indirect pathway includes the external segment of the globus

pallidus (GPe), which connects the striatum to the output regions of the basal ganglia (Haber et al. 1993; Francois et al. 2004). It is therefore likely that the ventral pallidum (VP), that is, the ventral part of the GPe in the primate, plays an important role in implementing the motivational functions of the indirect pathway. Given its connection with the ventral striatum (VS; Parent 1990; Parent and Hazrati 1995; Spooren et al. 1996; Smith et al. 1998), the VP may be involved in appetitive or aversive motivations, selection of their associated behaviors, the appetitive approach, or avoidance of aversive behaviors.

Human studies have identified components of the basal ganglia, such as the VS, as critical contributors to aversive as well as appetitive learning (Jensen et al. 2003; Nitschke et al. 2006; Delgado et al. 2009; Pohlack et al. 2012; Bolstad et al. 2013). Nonhuman primate studies that associate neuronal recording with delayed response task, where monkeys actively engaged in goal-directed behavior, have primarily focused on the appetitive domain and consistently shown reward anticipatory activity in the VS (Hollerman et al. 1998, 2000) and the VP (Tachibana and Hikosaka 2012). Only one study was conducted to examine the GPe under Pavlovian conditioning in which monkeys passively engaged in the task with appetitive and aversive outcomes (Joshua et al. 2009). These human and nonhuman studies confirm that the basal ganglia, and particularly the interconnected VP and VS, translate positive motivation into appetitive action (Mogenson et al. 1980; Smith et al. 2009). Local interference with the VP function through the injection of bicuculline (a $GABA_A$ antagonist) induces stereotyped behaviors (i.e., repetitive finger biting and compulsive grooming), which could reflect a stressful or an anxious state that underlies the avoidance behavior (Grabli et al. 2004). However, it has remained largely unclear whether and how the VP contributes to processing negative motivational states (CS-based aversive behavior and anticipation of aversive outcomes) under a context in which subjects could avoid an aversive outcome (active avoidance behavior).

Using 2 variants of the delayed response task, we investigated the hypothesis that the VP is involved not only in appetitive behavior but also in aversive behavior, particularly active avoidance and escape behavior. To prove this hypothesis, we injected bicuculline, which is a $GABA_A$ antagonist that blocks inhibitory striatal afferents and local inhibitory interactions between pallidal neurons (Matsumura et al. 1995; Kita et al. 2004). Blocking these inhibitions leads to the disruption of information transmission (inside the indirect pathway) and abnormal increased activity of VP neurons. Bicuculline injections induced nonadaptive avoidance behavior (i.e., escape behavior), particularly when the monkeys anticipated an aversive outcome and an increase of heart rate. Moreover, using single-cell recording, we found that VP neurons were modulated not only by appetitive events but also by aversive ones, particularly during the presentation of an aversive CS and during the anticipation of aversive outcomes. These results revealed that the VP plays a crucial role in controlling negative motivation to produce active avoidance. Moreover, they suggest that the disinhibition of the indirect pathway by activation of VP neurons enhances the encoding of aversive stimuli and the anticipation of aversive outcomes, which may result in a change in the physiological and emotional states and escape behaviors, as found in patients with various psychiatric disorders.

Materials and Methods

A female rhesus monkey (*Macaca mulatta*, weighing 5 kg; Monkey T) and a male fascicularis monkey (*M. fascicularis*, weighing 4 kg; Monkey C) were used in this study. Animal care and housing were in accordance with the guidelines of the National Institutes of Health (1996) and the recommendations of the European Communities Council Directive of 2010 (2010/63/UE) and the French National Committee (87/848).

Apparatus

During the experimental sessions conducted in a dark room, the monkeys were made to sit in a chair with their heads fixed. A metal bar with a touch sensor was installed at waist level in front of the chair, which the monkeys could easily hold and release with their left hand. A 19-inch color video monitor equipped with a touch-sensitive screen was placed in front of the monkey (27 cm from the eyes). Eye movements, eye positions, and blinking were monitored at 120 Hz using an infrared eye-tracking system (resolution, 0.25° visual angle; DQW-1 version 1.20; ISCAN, Inc., MA, USA). Licking was detected whenever the tongue interrupted an infrared beam installed in the juice delivery system. The behavioral data and neuronal data were collected at 1000 Hz with a Spike2 data acquisition system (Cambridge Electronic Design Ltd, Cambridge, UK). Presentation (Neurobehavioral Systems, Inc., CA, USA) and Scenario manager (Institut des Sciences Cognitives, Centre de Neuroscience Cognitive, Bron, France) software were used to control the behavioral task, along with solenoid valves that opened and closed the reward delivery system and the air-puff system. Single drops of 0.2 mL of apple juice served as a reward and were delivered via a small plastic hole placed in front of the monkey's mouth. Single puffs of air delivered at 1.5–2.0 bar (25–35 psi) served as punishment. They were directed to the left side of the monkey's face, including the cheek and eye, and delivered through a tube with its opening set at a distance of 10–15 cm from the face.

Surgery

After the monkeys had learned the task, we implanted the head fixation system and a chamber to record neuronal activity. Aseptic surgery was performed under isoflurane anesthesia. Antibiotics and analgesics were used to prevent postsurgical infection and pain. Plastic and titanium screws were implanted in the skull, and the head fixation ring was attached using acrylic resin. Part of the skull over the right frontal lobe was removed, and a recording chamber was implanted to permit access to the anterior part of the VP and of the striatum. To measure heart rate during the task, we implanted a cardiac beat measurement instrument (Data Science International) under the left axillary skin in Monkey T.

Behavioral Tasks

To provide certain predictable contexts, the delayed response tasks consisted of a single- (Fig. 1A) and dual-cue variant (Fig. 1B), which were presented in alternating blocks (see below). The appetitive and aversive single-cue tasks allowed us to separate positive context-related activity from negative context-related activity, whereas the dual-cue task enabled us to investigate the nature of the value signals processed by the anterior VP.

To start a trial in either task, the monkeys had to hold the bar with their left hand (Fig. 1A). A small white dot (starting point, visual angle of 0.4°) immediately appeared at the center of the screen. After 1.3 s, it was replaced by 1 (single-cue task) or 2 (dual-cue task) CSs (visual angle of 11°). CSs were presented for 1.0 s pseudorandomly on the left or right side (single-cue task) or on both sides (dual-cue task) of the touch screen. The CSs

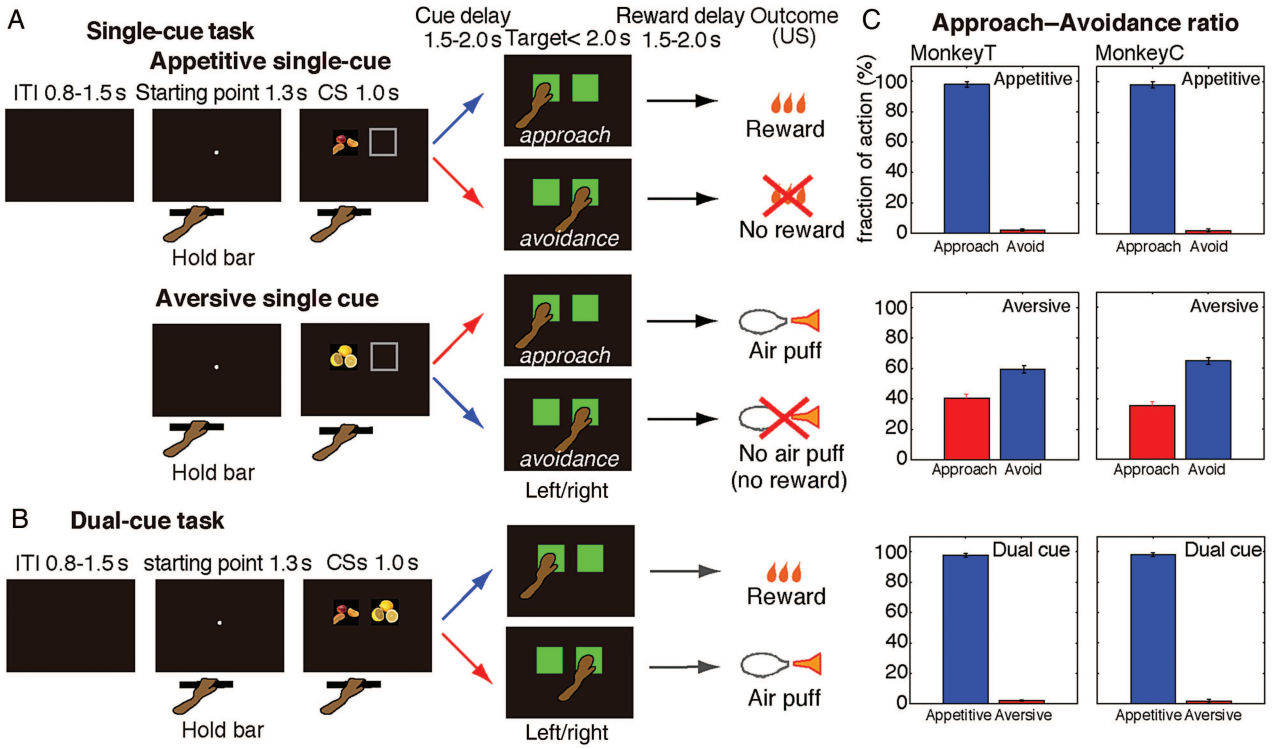

Figure 1. Delayed response tasks, task schedule, and behavioral results. (*A*) Single-cue task: appetitive (top) and aversive single-cue task (bottom). A trial was started by holding the bar and the appearance of a white starting dot. After the starting point had been presented for 1.3 s, one of the CSs was presented in the left or right position. Two green targets appeared after a delay for choosing an action, either approach or avoidance behavior, leading to positive US (a drop of juice), negative US (a puff of air), or no US. (*B*) Dual-cue task. The temporal sequence was the same as in (*A*). Two CSs were presented, and the monkeys chose one of them to obtain the US associated with it. (*C*) Approach and avoidance behavior during the recording sessions. The bars indicate the average percentages of approach with SEM in appetitive single-cue trials and avoidance in aversive single-cue trials for each monkey. In the dual-cue task, the bars indicate the average percentages of selecting the target associated with the appetitive or aversive CS.

were appetitive or aversive, that is, they were associated with specific contexts in which the monkeys have a chance to obtain liquid reward or air-puff punishment as USs. After the CSs had disappeared, a random delay period of 1.5–2.0 s occurred.

Then, green square targets (visual angle of 12°) were presented for a maximum of 2.0 s on the left and right sides of the screen. In both tasks, monkeys had to select 1 of the 2 targets by touching the screen. In the single-cue task, selecting the target in the same position in which the CS had been presented corresponded to approach, whereas selecting the target in the other position corresponded to avoidance. The targets disappeared as soon as one of them had been selected. If the monkeys selected the target at the position where the CS had been demonstrated, the liquid reward (appetitive CS approached) or the air-puff (aversive CS approached) occurred after a random delay of 1.5–2.0 s. In contrast, if they selected the target at the other position nothing happened, that is, the monkeys missed out on the opportunity to earn a reward (appetitive CS avoided) or successfully prevented an air-puff (aversive CS avoided). In the dual-cue task, the outcome associated with the CS that was presented at the selected target position was delivered. Thus, the monkeys chose to approach or avoid in the single-cue task depending on the appetitive or aversive nature of the previously presented CS, and they selected the target that appeared in the position of the preferred CS in the dual-cue task. In both tasks, trials were separated by an intertrial interval (ITI) of 0.8–1.5 s. To maintain the motivation of the monkeys to perform in the single-cue task, the aversiveness of the air-puff had to be limited and aversive trials occurred only after appetitive trials. Monkeys were never punished by air-puff if they failed to make a complete trial (i.e., error); instead, aversive trials were repeated. Thus, they were allowed to escape (i.e., making error) from tasks without punishment, but they had to complete an aversive trial to perform the next appetitive trials.

Three different types of errors could occur. First, trials in which monkeys released the bar prematurely, that is, before CS presentation and during CS presentation, were categorized as premature responses. Premature responses before and during CS presentation could be interpreted as excessive anticipation for an upcoming CS presentation and excessive reaction to the CS (hypervigilance). Second, trials in which monkeys produced no response at all during the 2-s target presentation were categorized as omissions, suggesting the loss of motivation to perform tasks or abnormal reaction to a trial-like freezing. The third error category consisted of trials in which monkeys touched outside of the target area, raising a movement problem. After a premature response, the trial was stopped and the CS(s) disappeared. In addition, after all the 3 error types, an identical trial was started upon the detection of the error and after a standard ITI.

To control for the possibility that anterior VP neurons respond to specific visual features, we used different CS images that we associated with specific contexts. In particular, we used 2 sets of abstract object images: 1 set of food images and 2 sets of social images (monkey faces and social interactions between monkeys). Different CS images were presented in groups of 10 trials. Monkeys viewed at least 3 sets of images per sub-block. The number of trials in a block was adjusted to the average performance level of each monkey. In particular, a block of the dual-cue task

consisted of 30 trials (for Monkey C) or 40 trials (for Monkey T). A block of the single-cue task consisted of 35 trials (for Monkey C) or 50 trials (for Monkey T). In the single-cue task, 60% (i.e., 21 or 30) of the trials were appetitive and 40% (i.e., 14 or 20) were aversive trials. To implement these proportions, no more than 2 appetitive trials were presented consecutively in the single-cue task. Thus, in the single-cue task, the monkeys were able to predict with certainty that the next trial will be appetitive after an aversive trial and that the next trial will be aversive after 2 consecutive appetitive trials, but they were uncertain about the next trial after an appetitive trial that was preceded by an aversive trial (see Supplementary Fig. 3).

Physiological Recordings

Neuronal activity was recorded using epoxy-insulated tungsten microelectrodes (FHC, Inc.; resistance: 2–4 MΩ at 1 kHz) inserted into the brain through a 23-gauge guide tube that penetrated the dura mater. The electrode approached 30° obliquely from the sagittal axis in Monkey T and vertically in Monkey C. A mechanical microdrive (NAN-A, Nan Instruments Ltd) was used to move the electrode in micrometer steps. Single-unit potentials were amplified using a multichannel processor (Plexon, Inc., Dallas, TX, USA).

To approximately localize the anterior pallidum in each monkey, we acquired magnetic resonance (MR) images (1.5 T, Sonata; Siemens) of the brain and the recording chamber (see Supplementary Fig. 1). The MR images provided us with an estimate of the location and depth of the pallidum with respect to the cortical surface and of the structures that the electrode would pass through before reaching the VP. We used a grid system, which allowed us to access the VP in intervals of 1 mm. The VP was investigated at anterior commissure (AC) + 1 and AC0. The average spontaneous activity of task-related neurons was 22.9 ± 13.6 spikes/s, which is consistent with previous reports (Tachibana and Hikosaka 2012), allowing us to distinguish them from the lower baseline activity neurons of other structures, such as the striatum. During the experiments, the AC could be identified as a silent region, which was the primary landmark for separating the dorsal and ventral parts of the anterior pallidum.

Bicuculline Microinjections

Bicuculline or saline was injected into the VP with a 30-gauge cannula tube connected to a 10-µL microsyringe (Hamilton). Each injection consisted of 1.5 µL of sterile bicuculline methiodide (Sigma) at a concentration of 15 µg/µL (29.5 mmol/l; Grabli et al. 2004) or saline (Aguettant). Prior to the initiation of the injection experiments, we roughly checked the position of the striatum and anterior pallidum (see Supplementary Fig. 1). Subsequently, VP neurons were identified several times along with confirming the dorsal and ventral borders of the anterior pallidum based on the higher baseline firing rate of VP neurons (see Supplementary Fig. 1A). The precise injection sites were based on the locations at which we had recorded task-related activities. Once the cannula arrived at the target position, the monkeys performed a preinjection session (2 sets of 35–50 trials of the single-cue task and 30–40 trials of the dual-cue task). Then, an experimenter entered the experimental room and injected bicuculline or saline at a speed of 1.0 µL/min in steps of 0.5 µL. In order for the substance to influence neuronal activity, behavioral testing continued for only 5 min after the end of the injection. We assessed the performance in the behavioral task for at least 1 h. During this time, the cannula was left at the injection site to minimize leakage outside of the target structure and to prevent the backflow of substances. Injections were performed 2 times per week at the most. The remaining schedule was allotted to neuronal recording or a behavioral control session without recording. The injection periods were defined corresponding to the time after injection; P1: 5–25 min, P2: 25–45 min, and P3: 45–65 min after injection.

Data Analysis

Behavioral Analysis

To quantify the behavioral performance during both injection and recording sessions, we counted how often the monkeys showed approach, avoidance, or 1 of the 3 error types (premature responses, omissions, and touching outside of the target area). To fully characterize how bicuculline affected premature responses, we classified the responses into 2 subgroups, depending on when they occurred in the trial: (1) pre-CS, if the monkeys released the bar already during presentation of the starting point at the beginning of a trial and (2) peri-CS, if the premature bar release occurred during CS presentation.

In addition, we analyzed the reaction time (RT), movement time (MT), and spatial response bias. The RTs and MTs were calculated as the time intervals from the target onset to bar release and from bar release to touching of the screen, respectively. The spatial response bias was determined separately for each context (appetitive and aversive) and task (single-cue and dual-cue) and separately for control sessions (preinjection) and injection sessions. It was calculated as follows: (number of left responses – number of right responses)/(number of left responses + number of right responses). The responses to the right are ipsilateral to the injection, whereas those to the left are contralateral to the injection. A bias of 1 means that the monkeys went left/contralateral on all trials, 0 means they responded evenly, and −1 means that they went right/ipsilateral on all trials.

Blinking

To estimate how much the monkeys anticipated the aversive outcome (air-puff), we calculated the number of blinks. We used the vertical component of the eye movement trace to detect blinks (Matsumoto and Hikosaka 2009). In particular, we set a threshold and calculated the downward movement of the eyelid during pre-CS presentation. We defined a blink as crossing the threshold within 500 ms and calculated how many times the eyelid crossed the threshold. Blinking was analyzed for both the whole task period and particularly for the 500 ms before US delivery, because monkeys could estimate the time of the air-puff based on the time they touched the target.

Licking

To determine how much the monkeys anticipated the appetitive outcome (liquid reward), we measured predictive licking behavior. In particular, we counted the number of times the tongue interrupted the infrared sensor during the 500 ms before the time of US delivery.

Heart Rate

Heart rate is one of the important and reliable physiological markers of negative emotional state (Hofmann et al. 2005; Fisher and Newman 2013). To monitor the physiological state during normal and bicuculline injection into the VP, the heart rate was assessed with an electrocardiogram and corresponded to average beats per minute (bpm) in single- and dual-cue tasks, during control sessions and injection sessions.

Neuronal Analysis

The activity of neurons was first plotted by spike density functions and histograms (Gaussian kernel, $\sigma = 10$ ms). Only

completed trials were kept in the database (i.e., trials in which the monkeys touched one of the targets on the screen within 2 s after the target onset). Moreover, we excluded neurons with unstable recording or insufficient trials (fewer than 8 trials for both appetitive and aversive trials in the single-cue task) from further analysis.

To characterize how VP neurons are modulated by different events within a trial, the activity was assessed in distinct 200-ms periods. In particular, we analyzed pre-CS activity in the 200-ms period before CS appearance, CS activity in the 201- to 400-ms period after CS presentation, target expectation activity in the 200-ms period preceding the presentation of the target, movement-related activity in the 200-ms period before the monkeys touched the target, US expectation activity in the 200-ms period prior to US delivery, and US receipt activity in the 51- to 250-ms period after the aversive US and the 201- to 400-ms period after the appetitive US (air-puff responses were typically more rapid than the responses to a liquid reward; Matsumoto and Hikosaka 2009).

To detect differential encoding of aversive and appetitive information in the VP, we performed three-way analyses of variance (ANOVAs; $P = 0.01$) using the following factors for the single-cue task: motivational context (appetitive vs. aversive), image (abstract images vs. food vs. social), and position (left vs. right). On the basis of this analysis, the activity in each period was classified into 1 of the 3 categories: (1) context-selective (Context < 0.01; Image ≥ 0.01; Position ≥ 0.01); (2) image-selective (Context ≥ 0.01; Image < 0.01; Position ≥ 0.01); and (3) position-selective (Context ≥ 0.01; Image ≥ 0.01; Position < 0.01); context- and image-selective (Context < 0.01; Image < 0.01; Position ≥ 0.01); context- and position-selective (Context < 0.01; Image ≥ 0.01; Position < 0.01); image- and position-selective (Context ≥ 0.01; Image < 0.01; Position < 0.01); and all (Context < 0.01; Image < 0.01; Position < 0.01).

To precisely characterize the activity of VP neurons during the pre-CS period, we grouped trials according to 3 situations, that is, uncertainty about the next trial (uncertainty), certainty of the next trial being appetitive (appetitive certainty), and certainty of the next trial being aversive (aversive certainty). These situations were compared with the two-tailed *t*-tests ($P < 0.01$, Bonferroni-corrected). The VP neurons were classified into 3 different categories in which the following criteria were fulfilled: (1) uncertainty vs. appetitive certainty < 0.01; (2) appetitive certainty vs. aversive certainty < 0.01; and (3) uncertainty vs. appetitive certainty < 0.01 and appetitive certainty vs. aversive certainty < 0.01.

Results

We tested the behavioral effects of bicuculline by injections into the VP with 2 different tasks (Fig. 1A,B). Subsequently, to characterize the temporal profile of aversive responses in the VP, we recorded the activity of VP neurons. In the single-cue task, we presented only one CS in each trial. The CS was used providing for a positive or negative context. The monkeys could then select whether to approach or avoid the CS (Fig. 1A, single-cue task). In the dual-cue task, we presented both appetitive and aversive CSs, which allowed us to characterize the precise nature of CS-induced activity in the VP (Fig. 1B, dual-cue task).

Behavioral Results

Both monkeys learned about the meaning of the different CSs and performed consistently in the different tasks (Fig. 1). We first counted the number of completed trials and incomplete trials (error trials). The monkeys completed >98% of the appetitive trials in the single-cue task and all the trials in the dual-cue task. In contrast, they completed 92 ± 11% (mean ± SD; Monkey C) and 81 ± 13% (Monkey T) of the aversive single-cue trials, resulting in a significant difference between the aversive single-cue trials and both the appetitive single-cue trials and the dual-cue trials ($P < 0.05$, two-tailed *t*-tests). Among the completed trials, the predominant behavior was approached in the appetitive single-cue trials (>95% of the completed trials, Fig. 1C: appetitive single-cue) and in the dual-cue task (Fig. 1C: dual-cue task). On the other hand, the monkeys avoided the target associated with an aversive US in >60% of the completed trials in the aversive single-cue trial (Fig. 1C: aversive, $P < 0.03$, binomial test for difference from 50%). Although the motivational value difference between the approach and avoidance of air-puff seems to be small, the highest proportions of avoidance in the aversive single-cue task were 84% in Monkey T and 78% in Monkey C during the recording sessions. This behavioral result indicates that there was a clear differential value between approach and avoidance of air-puff in the aversive trial. Significantly different proportions of approach and avoidance were observed between appetitive and aversive single-cue trials ($P < 0.01$, χ^2 test), but not between the appetitive single-cue trials and the dual-cue trials ($P > 0.05$, χ^2 test). Thus, as would be expected, the monkeys primarily approached the appetitive targets and avoided the aversive ones, suggesting that different CSs provided monkeys with positive and negative contexts.

On the basis of proportions of approach and avoidance responses, one may ask whether the aversive cue was processed at all and whether the aversive single-cue trials were simply less appetitive than the appetitive ones. We therefore performed a more detailed analysis of incomplete trials (errors), response timing, and licking and blinking behaviors. We classified the errors into 3 different types: premature response, touching outside of the target, and omission (see Materials and Methods). Both monkeys showed significantly more errors in aversive single-cue trials than in appetitive single-cue trials and in dual-cue trials, irrespective of error type, except for omissions in Monkey C (premature responses, mean ± SEM: Monkeys T/C: 5 ± 1%/5 ± 1%, both $P < 0.01$; outside of the target: 7 ± 1%/2 ± 1%, both $P < 0.05$; omissions: 7 ± 1%/1 ± 0.2%, $P < 0.01$ in Monkey T, but $P > 0.05$ in Monkey C, two-tailed *t*-tests). All these errors were nonadaptive, because the monkeys had to perform an identical trial again whenever they made an error in a trial.

The RTs and MTs in aversive single-cue trials were significantly longer than those in appetitive single-cue trials or dual-cue trials, irrespective of approach or avoidance behavior (Fig. 2A,B, $P < 0.001$, for RTs in both monkeys; $P < 0.001$ for MTs in Monkey T, $P < 0.05$ for MTs in Monkey C, two-tailed *t*-tests). Thus, the monkeys discriminated among the different CSs and outcomes. Furthermore, both monkeys showed significantly more licking during the outcome anticipatory period (i.e., reward delay) in both the appetitive single-cue trials and the dual-cue trials compared with the anticipatory period in the aversive single-cue trials (Fig. 2C, $P < 0.01$ in comparison with appetitive trials and dual-cue trials, two-tailed *t*-tests). Finally, both monkeys showed significantly more blinking in aversive single-cue trials when they approached an aversive target than when they avoided it during the outcome anticipatory period (Fig. 2D, $P < 0.01$, two-tailed *t*-tests for appetitive single-cue trials and dual-cue trials), indicating that the monkeys anticipated different outcomes. Taken together, these behavioral results suggest that the monkeys processed aversive single cues, they processed these cues differently from appetitive single-cue and dual-cue tasks, and they anticipated different outcomes depending on whether they approached or avoided aversive single cues.

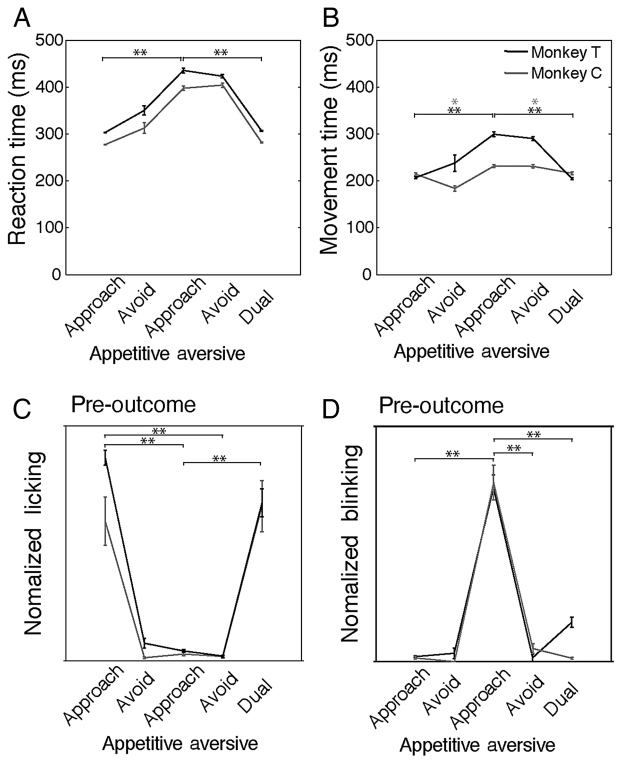

Figure 2. RT, MT, licking, and blinking in the pre-outcome period. (*A*) RT (mean ± SEM; ms) of both monkeys in each task. Black and gray lines indicate the results for Monkey T and Monkey C, respectively. Black asterisks indicate significant differences in both monkeys (two-tailed *t*-tests, $^{*}P < 0.05$, $^{**}P < 0.01$). (*B*) MT. Black and gray asterisks indicate significance in Monkeys T and C. (*C*) Normalized licking in the pre-outcome period. The asterisks indicate the same as in (*A*). (*D*) Normalized number of blinks in the pre-outcome period. Asterisks indicate the same as in (*A*).

Bicuculline Microinjections into the VP Disturb Avoidance Behavior

To examine how the VP contributes to the control of avoidance (and approach) behavior in aversive (and appetitive) contexts, we injected bicuculline into the VP (1.5 µL, concentration of 15 µg/µL) while the monkeys performed the tasks. Following previous studies (Grabli et al. 2004; Tachibana and Hikosaka 2012), we used the AC as a landmark for separating the VP from the dorsal pallidum in the ventrodorsal direction (Fig. 1). We made 12 bicuculline injections in the VP ($n = 7$ in Monkey T and $n = 5$ in Monkey C) at 3 different AP levels, that is, 1 mm anterior to the AC (AC + 1), at AC, and 1 mm posterior to the AC (AC – 1, Fig. 3*A*). Before the injections, the performance was similar as in the recording sessions (Fig. 3*C*,*E*). Moreover, after the injections, the monkeys showed no significant increase in the rate of touching outside of the target ($P > 0.05$, two-tailed *t*-tests; Fig. 4*C*,*D*). These data indicate that the sensory motor processes were largely unaffected.

Importantly, bicuculline induced various behavioral effects 5–25 min (10 sessions; Fig. 3*A*, red sites) or 25–45 min (2 sessions, Fig. 3*A*, yellow sites) after the injection. In particular, the number of incomplete trials was significantly increased after bicuculline injections, especially in the aversive single-cue trials (Fig. 4*D*,*F*, $P < 0.05$, compared with appetitive single-cue trials and dual-cue trials, two-tailed *t*-tests). Thus, bicuculline injections significantly increased the number of errors in the aversive single-cue trials.

The increase in incomplete trials after bicuculline injection in the VP was primarily due to an increase in premature responses (Table 1 and Fig. 4*E*,*F*). Premature responses increased most strongly in aversive single-cue trials ($P < 0.001$ in Monkey T, $P < 0.005$ in Monkey C, two-tailed *t*-tests compared with preinjection sessions). In the dual-cue task, they significantly increased in Monkey T ($P < 0.05$, two-tailed *t*-test, compared with preinjection sessions) but not in Monkey C ($P = 0.167$, two-tailed *t*-test). Both monkeys also showed a moderate increase in premature responses in the appetitive single-cue trials (Fig. 4*E*,*F*; appetitive single-cue task), which was significant in Monkey C ($P < 0.05$) and significant only during 25–45 min after injection in Monkey T ($P < 0.05$, two-tailed *t*-test). The omissions in aversive single-cue trials increased primarily in Monkey C ($P < 0.02$, two-tailed *t*-test; Monkey T: $P = 0.789$, two-tailed *t*-tests).

Interestingly, both monkeys showed a reduction of successful avoidance behavior in aversive single-cue trials (Fig. 4*B* middle, $P < 0.01$ in Monkey T, $P < 0.03$ in Monkey C, two-tailed *t*-tests), but no significant change in approach behavior in appetitive single-cue trials ($P = 0.0892/0.1995$ in Monkeys T/C, two-tailed *t*-tests). Thus, bicuculline impaired the avoidance of aversive outcomes and increased the number of premature responses and omissions, particularly in aversive single-cue trials.

During the injection experiments, we also observed that the monkeys chose the target presented on the left side (contralateral to the injection sites) more often than the target presented on the right side, especially in aversive single-cue trials (spatially biased response to the contralateral side; see Supplementary Fig. 2; $P < 0.01$, two-tailed *t*-tests compared with preinjection sessions in both monkeys). Although significant spatial response biases were also seen in appetitive single-cue trials in Monkey C ($P < 0.03$ in Monkey C, $P = 0.0501$ in Monkey T, two-tailed *t*-tests), as well as in dual-cue trials ($P < 0.03$ in Monkey T, but $P = 0.1427$ in Monkey C), the spatial bias was significantly stronger in aversive single-cue trials than in appetitive single-cue trials (see Supplementary Fig. 2; $P < 0.001$ in Monkey T, $P < 0.02$ in Monkey C, two-tailed *t*-tests).

In separate control sessions, we made bicuculline injections into the internal segment of the globus pallidus (GPi, Fig. 3*A*, 3 injections) or saline injections into the VP (6 injections; 4 in Monkey T and 2 in Monkey C). In these injections, we observed no significant modification of premature responses, omissions, or spatial biases (Fig. 4*C*,*D*: Control). Therefore, these behavioral effects were induced exclusively by VP injections. Overall, the behavioral effects suggest that bicuculline injection into the VP increased unsuccessful attempts to avoid an aversive outcome, particularly in the aversive single-cue context that could lead to a negative outcome.

Bicuculline Microinjections into the VP Increase Nonadaptive Avoidance of Aversive Outcome

One of the more remarkable effects of bicuculline injections into the VP was the increased premature responses, particularly in aversive single-cue trials. These premature responses could reflect excessive anticipation of aversive CSs (in the pre-CS period) and/or enhanced reaction to aversive CSs (in the peri-CS period). To assess these possibilities, we analyzed the timing at which these premature responses occurred and found that they occurred during all phases (Fig. 4*E*,*F*), specifically for aversive single-cue trials when compared with appetitive single-cue trials and with dual-cue trials ($P < 0.02$, two-tailed *t*-tests).

Given these findings, an obvious follow-up issue is how the monkeys can possibly know about the upcoming CS and make

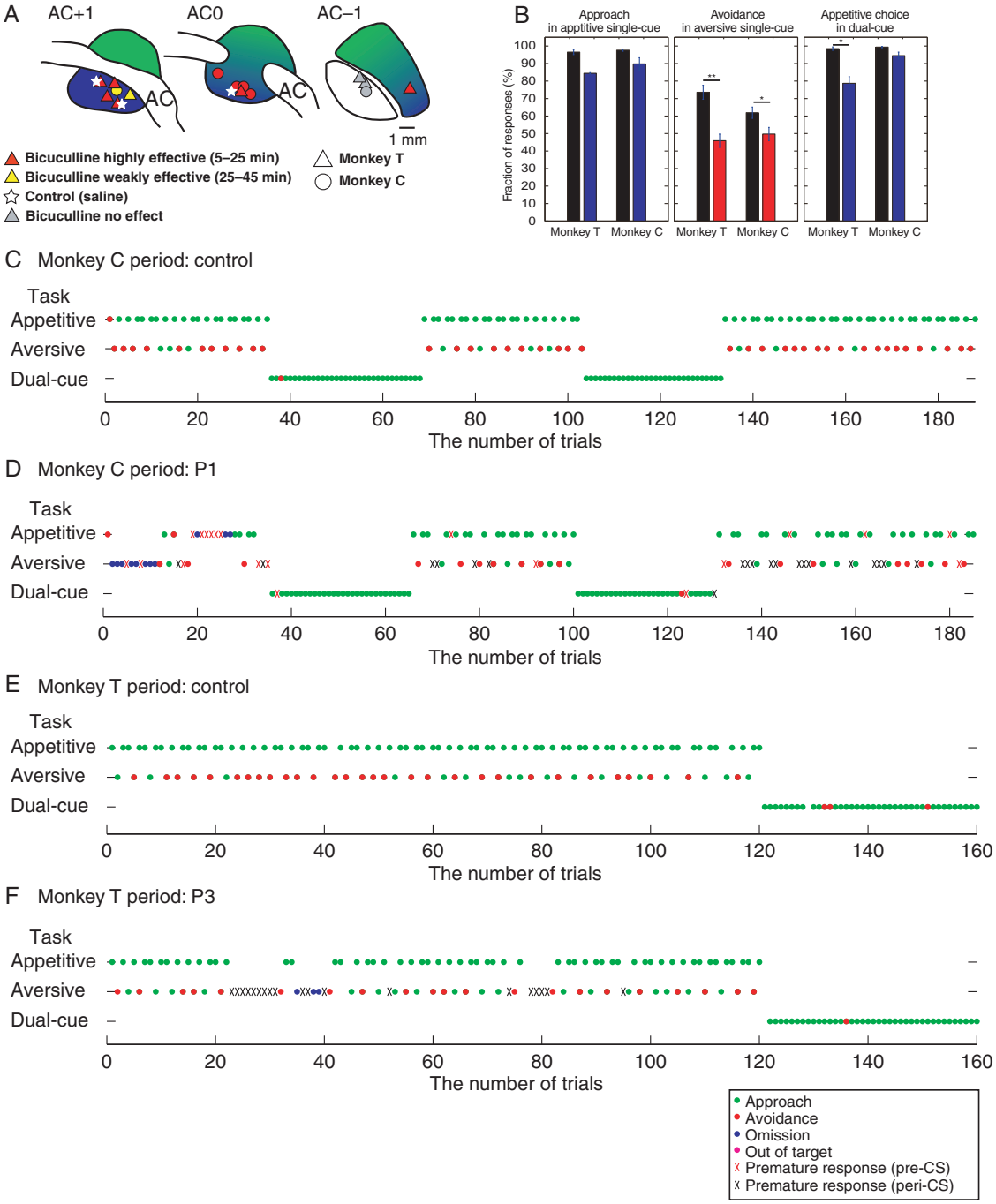

Figure 3. Bicuculline injection sites, the fraction of approach–avoidance, and choice patterns pre- and post-injection. (*A*) Reconstruction of injection sites. AC + 1 represents 1 mm anterior to the AC, at the AC (AC0), and 1 mm more posterior to it (AC – 1). Red and yellow marks represent the latency of the injection effect, and the shape indicates the subjects. White stars represent the control sessions with saline. The gray marks represent the injection site of the GPi with no effect. The blue and green zones represent limbic and associated functional territory, respectively, based on previous studies (Francois et al. 2004; Grabli et al. 2004). (*B*) The fraction of approach in appetitive single-cue trials, that of avoidance in aversive single-cue trials, and that of appetitive choice in dual-cue trials. Black bars indicate performance preinjection sessions, and color bars indicate post-injection sessions. $^{*}P < 0.05$, $^{**}P < 0.01$. (*C*–*F*) Behavioral choice patterns preinjection (*C*,*E*) and post-injection (*D*,*F*). Each plotted mark represents behavioral choice in a trial.

more premature responses before an aversive CS, given that they have not even seen the CS yet. When the monkeys failed to make a correct response (i.e., an error trial), an identical trial was repeated. Thus, after an error trial, the monkeys could predict the next trial. We therefore expected an increased prevalence of premature responses during the pre-CS period of aversive single-cue trials. This would be due to an increased prevalence of committing premature responses after a previous error, and we found that this was indeed the case. Figure 4G shows that consecutive premature responses were observed significantly more frequently in post-injection than in preinjection sessions ($P < 0.01$ in Monkey T, $P < 0.05$ in Monkey C, two-tailed *t*-tests). It is worth noting that these consecutive premature responses were nonadaptive because they prevented the monkeys from

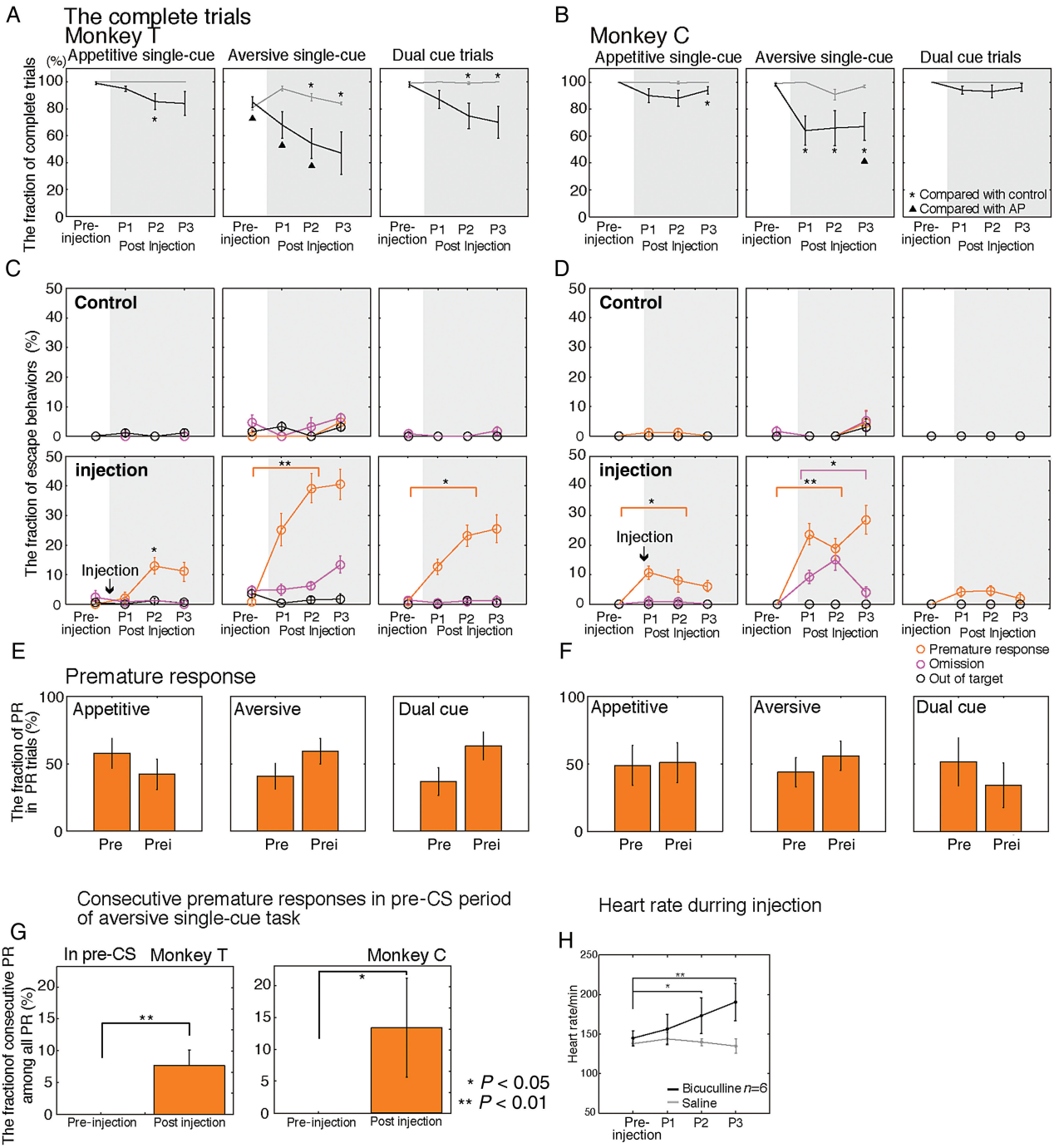

Figure 4. Temporal structure of behavioral effects and heart rate. (A,B) The proportion of complete trials. The gray lines represent performance in the control sessions, and the black lines indicate the injection sessions. White and gray areas correspond to pre- and post-injection phases. Stars and triangles in each panel indicate significance compared with control or appetitive single-cue trials ($P < 0.05$). The P1, P2, and P3 in horizontal axis. (C,D) Errors in control sessions top; in Monkey T (C, $n = 4$) and Monkey C (D, $n = 5$) and bicuculline sessions [bottom; Monkey T ($n = 7$) and Monkey C ($n = 5$)]. Orange, magenta, and black marks show nonadaptive behaviors: premature responses, no response (omissions), and touching outside of the target, respectively. (E,F) Proportion of premature responses in each task. Premature responses before (pre) versus during (peri) presentation of CS are shown for Monkey T (E) and Monkey C (F). (G) Proportion of consecutive premature responses in the pre-CS period of the aversive single-cue trials during pre- and post-injection, including P1–P3 (*$P < 0.05$, **$P < 0.01$). (H) Heart rate in pre- and post-injection phases. A significant increase of heart rate was observed after P2 ($P < 0.03$) and P3 ($P < 0.01$).

performing trials with appetitive outcomes. Another possible interpretation of premature responses could be an increase of reward expectation that produces impulsive responses (i.e., the monkeys expect reward excessively and they were unable to wait for the appearance of the target). If that was the case, premature responses could be observed especially in appetitive contexts (appetitive single-cue and dual-cue trials) rather than for aversive single-cue trials. However, this was not the case. To go further into the hypothesis that premature responses can be due to an increase in the expectation of reward, we looked for behavioral markers that may indicate an expectation of reward. During control sessions, when the animals were highly

motivated, we observed 2 behavioral markers indicating a large appetitive motivation; completing their actions by touching the screen (although the targets are not displayed on the screen) and licking the rewards distributed before obtaining the reward. During the premature responses induced by bicuculline injections, the premature licking behavior was never observed for both monkeys, and only 9 and 14% (Monkeys C and T, respectively,) of premature responses observed in the appetitive contexts are terminated by screen touching. The large majority of premature responses were similar to those observed in the aversive context with withdrawal of the hand without touching the screen. Thus, the premature responses in appetitive contexts (appetitive single-cue trials and dual-cue trials) induced by bicuculline were unlikely reflected impulsive responses due to an increase of reward expectation.

Finally, we measured the heart rate of Monkey T while this monkey was performing the tasks during the injection sessions. The baseline heart rate was 142 ± 9 bpm (mean ± SD) and increased after injection to 151 ± 11, 164 ± 15, and 178 ± 24 bpm during P1, P2, and P3, respectively (Fig. 4*H*). Thus, the heart rate gradually and significantly increased in the bicuculline injection sessions ($P = 0.0134$ and 0.0067, two-tailed *t*-tests, injection after P2 and P3, respectively, $n = 6$), but not in the saline injection sessions ($P > 0.1$, two-tailed *t*-test, $n = 3$). The time course of heart rate increase correlated positively with the number of premature responses ($r = 0.3174$, Pearson's correlation), possibly reflecting the change of emotional state and anticipation of aversive events. Taken together, these results suggest that hyperactivation of the VP by bicuculline injection induced aberrant escape behaviors (i.e., premature responses and omissions). These abnormal behaviors should normally be controlled under the aversive context in order to execute active avoidance.

Table 1 Proportion of premature responses in pre- and post-injection sessions

Percentage	Preinjection	P1	P2	P3
Monkey T				
AP	0	2.8 ± 0.9	13.2 ± 0.5*	11.3 ± 6.4*
AV	0	27.3 ± 8.6**	38.9 ± 10.3**	40.5 ± 9.7**
Dual	0.8 ± 0.4	12.5 ± 5.8*	23.1 ± 7.0*	25.7 ± 5.9*
Monkey C				
AP	0	10.0 ± 4.2*	10.7 ± 5.3	5.7 ± 2.2*
AV	0	25.0 ± 6.7**	18.0 ± 7.4*	28.1 ± 8.8**
Dual	0	4.3 ± 2.9*	5.1 ± 3.6	2.8 ± 2.1

AP: appetitive single-cue trials; AV: aversive single-cue trials; Dual: dual-cue task.
*$P < 0.05$, **$P < 0.01$, compared with preinjection sessions.

VP Neurons Process Both Aversive and Appetitive Events

The bicuculline injections showed that abnormal activity in the VP induces monkeys to escape aversive contexts by committing more errors and completing fewer. These results raise questions about how VP neurons encode aversive and appetitive contextual information and whether the neuronal activity in the VP contributes to adaptive behavior in different motivational tasks. Because excessive CS-related activity and US-anticipatory activity could contribute to aberrant behavioral reactions to the CS and to inappropriate prediction of negative events, we expected that VP neurons would show strong activity related to aversive CSs and to the anticipation of aversive USs.

We recorded 162 neurons ($n = 76$ in Monkey T and $n = 86$ in Monkey C) in the VP with stable isolation and sufficient trials to analyze. In the posterior–anterior direction, the recording sites ranged from the level of AC + 1 to AC0 (Fig. 6*C*,*F* and see Supplementary Fig. 1). To examine whether and how VP neurons respond in the 2 different types of single-cue trials, we first analyzed all the recorded neurons by a three-way ANOVA ($P < 0.01$), using the 3 factors (aversive/appetitive, image, and position, see Materials and Methods). We found that the majority of neurons responded differently in aversive compared with appetitive single-cue trials (Table 2). A smaller number of neurons showed differential activity for the factors image, position, or multiple factors. Hereafter, we therefore focused on the neurons modulated by aversive or appetitive task events.

To avoid aversive outcomes and approach appetitive outcomes, the monkeys were required to discriminate CSs and to appropriately choose one of the targets. We first analyzed neuronal activity in the CS period (201–400 ms after CS appearance; see the section Neuronal Analysis), because the monkeys should discriminate between CSs during this time period. Interestingly, >75% of CS-related VP activity ($n = 40/53$; Table 2) distinguished between aversive and appetitive single-cue trials. We therefore classified neurons as negative context-preferring if their activity was stronger in aversive single-cue trials than in appetitive single-cue trials and vice versa for positive context-preferring neurons. Out of 40 neurons, 25 (63%) were positive context-preferring, showing excitation to an appetitive CS and suppression to an aversive CS (Figs 5A and 6A). The remaining 15 (37%) neurons were negative context-preferring and showed the opposite response pattern (Figs 5*B* and 6*B*). Although it was not significant, population activity in dual-cue trials shown in Figure 6A showed a tendency to reduce CS-related activity compared with appetitive one, suggesting that the existing of aversive CS modulates VP activity (average ± SEM: 26.8 ± 2.4 spikes/s in appetitive trials, 21.4 ± 2.1 spikes/s in dual-cue trials, $P = 0.1013$, two-tailed *t*-test). These neurons were distributed similarly across the

Table 2 Numbers of task-related neurons and preferential neurons

N = 162	Pre-CS ($n = 25$)	CS ($n = 61$)	Target ($n = 37$)	Movement ($n = 35$)	Pre-outcome ($n = 50$)	Post-outcome ($n = 57$)
Context only	12	40	23	23	42	51
Image only	11	8	8	5	7	5
Position only	1	5	0	5	1	0
Context × image	1	6	2	0	0	1
Context × position	0	2	3	2	0	0
Image × position	0	0	1	0	0	0
All	0	0	0	0	0	0
Positive context-preferring	7	25	15	15	20	26
Negative context-preferring	5	15	7	8	22	25

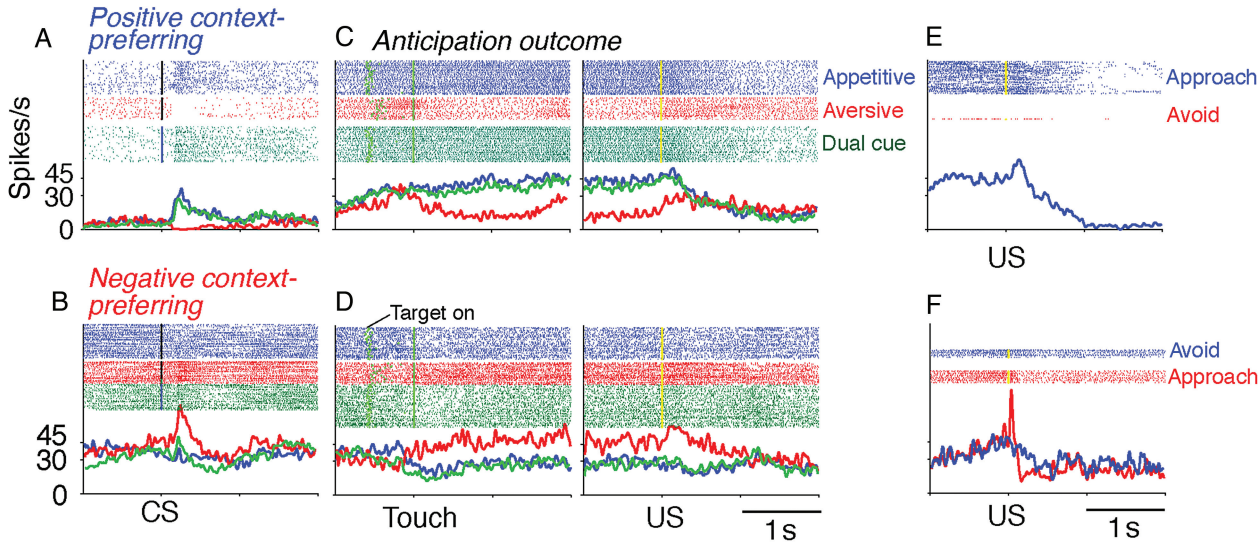

Figure 5. Examples of single neurons. (*A*) Neuron showing positive context-preferring CS-related activity. (*B*) Neuron showing negative context-preferring CS-related activity. (*C*,*D*) Different single neurons showing positive- and negative context-preferring US anticipatory activity (aligned to touching of the target on the left and to US onset on the right). (*E*,*F*) Different single neurons showing positive and negative context-preferring US-related activity. The raster displays and histograms represent the activity in appetitive single-cue trials (blue), aversive single-cue trials (red), and dual-cue trials (green).

mediolateral and dorsoventral extent of the VP (Fig. 6*C*). These findings suggest that the VP also responds to aversive events and that it discriminates between CSs based on their appetitive or aversive contexts.

Given that aversive single-cue trials primarily elicit avoidance whereas appetitive single-cue trials primarily elicit approach, we next investigated whether differences arising from these different behavioral responses would explain the activity of VP neurons. We therefore replaced the appetitive/aversive factor with an approach/avoidance factor and again performed a three-way ANOVA ($P < 0.01$) with the 3 factors: behavior (avoidance/approach), image, and position. This analysis was limited to aversive single-cue trials because the monkeys predominantly chose approach behavior in appetitive single-cue trials. Of the neurons showing CS-related activity, none was modulated by avoidance versus approach, and only one movement-related VP neuron showed such modulation. Thus, VP activity in the CS period appears to represent the appetitive or aversive contexts rather than the choice of the subject.

Anticipation and processing of USs is crucial for the appropriate preparation and response to positive and negative outcomes. We found VP neurons showing US-anticipatory activity or US-receipt activity (pre-US period: 200 ms prior to outcome delivery, that is, after the monkeys made a decision and were waiting for the outcome; Fig. 5*C*,*D*; for US-receipt activity, see Supplementary Text 1). Forty-two (26%) neurons showed US-anticipatory activity, which was either negative context-preferring or positive context-preferring (Fig. 6*D*,*E*). Thus, the VP encodes the anticipation of aversive and appetitive outcomes with excitatory and inhibitory activities.

Although US-anticipatory activity typically became differential for the appetitive or aversive nature of the outcome after the monkeys touched the screen, some neurons discriminated even earlier. We then performed a latency analysis of these US-anticipatory activities at single-neuron level and population activity level. The latency was determined as the time point where the neuronal activity exceeded 25% of the peak activity extracted from around reward delivery (500 ms pre- and post-outcome periods) from baseline activity (reference period was 500 ms before presentation of CS) in positive context-preferring and negative context-preferring neurons. At the single-neuron level, the latency for positive context-preferring neurons was 151.3 ± 193.0 ms (mean ± SEM) after touching the screen and that for negative context-preferring neurons was 133.5 ± 231.5 ms ($P = 0.6410$, *t*-test). However, at the level of population activity (Fig. 6*D*,*E*), the latency for positive context-preferring neurons was 279 ms before touching the screen and that for negative context-preferring neurons was 1272 ms after touching the screen (645 ms before outcome delivery). Thus, although the analysis of the activities at the population level shows that the latency of anticipatory activity in positive context-preferring neurons is faster than that of negative context-preferring neurons, the difference in latencies of activity of these neurons is not significantly different between aversive and appetitive trials at the single-neuron activity level.

The VP neurons encoding appetitive or aversive information showed more CS-related activity and US-anticipatory activity than movement-related activity (both $P < 0.03$, χ^2 test). Moreover, some of them encoded the upcoming outcome even before the monkeys selected 1 of the 2 targets and the activities were not movement-related. These results indicate that VP neurons predicted and anticipated both aversive and appetitive outcomes, without determining the associated behavior (approach or avoidance).

Finally, although the large majority of neurons had opposing activities between both motivational contexts, 9 neurons were commonly excited or inhibited by both aversive and appetitive events, possibly reflecting salience (see Supplementary Text 2). Thus, the VP preferentially encodes motivational contextual information rather than salience signals.

VP Activity Reflects Aversive Certainty and Uncertainty

VP neurons showed CS-related activity and US-anticipatory activity. However, activity at these time points cannot relate to premature responses during the pre-CS period and to consecutive premature responses, which were enhanced by bicuculline. The monkeys made these premature responses particularly when they were certain that an aversive trial would be the next trial,

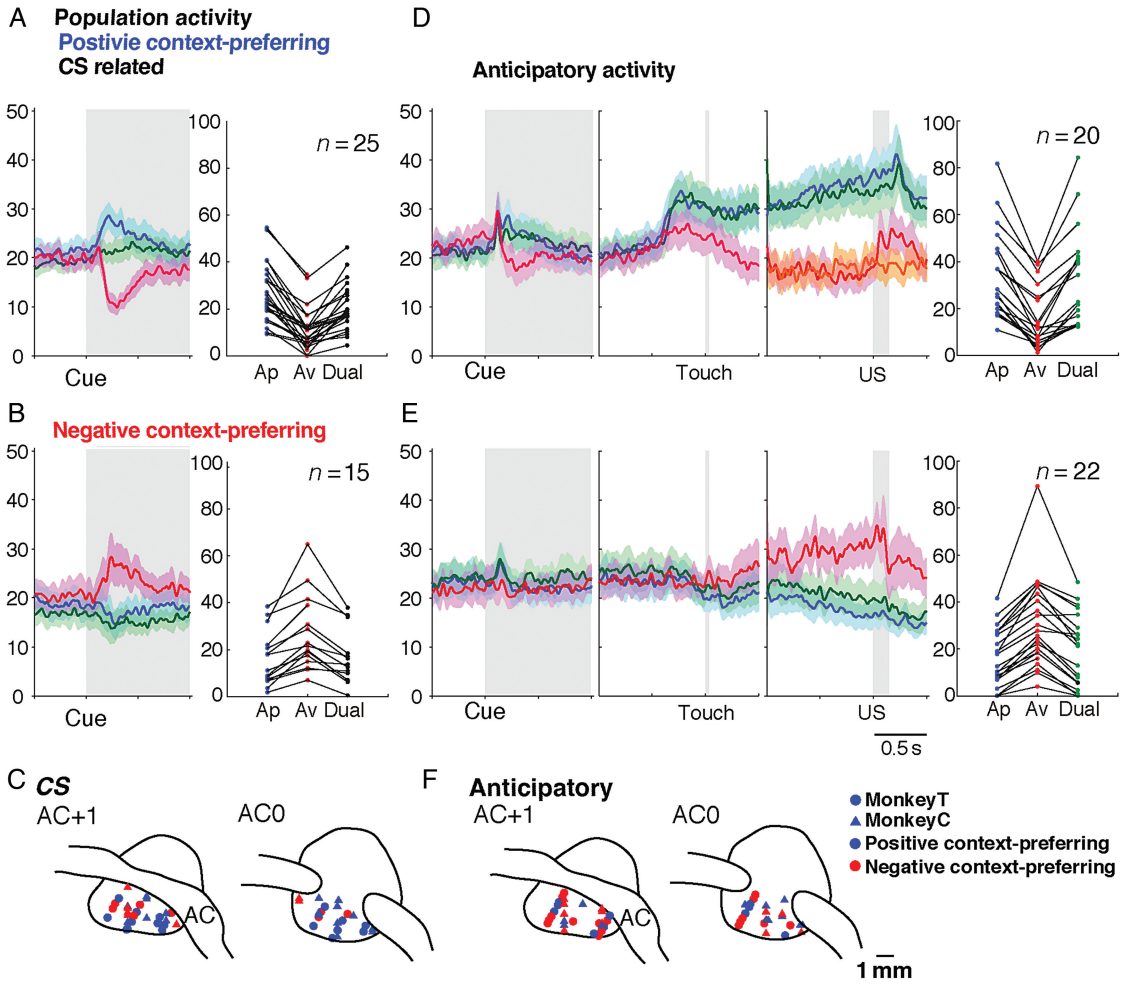

Figure 6. Average activity of populations with CS-related activity and US-anticipatory activity in appetitive single-cue trials, aversive single-cue trials, and dual-cue trials. (*A*,*B*) Population activity of positive context-preferring neurons and negative context-preferring neurons during the CS period. Gray area represents the CS period lasting 1 s. The activities in each trial (mean ± SEM) are shown by blue (appetitive single-cue), red (aversive single-cue), and green (dual-cue). The average activity of each individual neuron in each task is shown in the right columns. (*C*) Distribution of CS-related appetitive- and negative context-preferring neurons within the VP. (*D*,*E*) Population activity of positive context-preferring and negative context-preferring neurons during the US-anticipatory period. The activity when monkeys avoid the air-puff is represented by orange color in *D*, and that of when they received the air-puff is displayed by red. (*F*) Distribution of anticipatory appetitive- and negative context-preferring neurons within the VP. Ap: appetitive single-cue; Av: aversive single-cue; Dual: dual-cue task.

as evidenced by consecutive errors in aversive single-cue trials. We therefore investigated whether a subpopulation of neurons predicts the nature of the next trial or is modulated in the pre-CS period by uncertainty about the next trial. To do so, we took advantage of our task design in which aversive single-cue trials were never repeated (see Supplementary Fig. 3), except after an error. In addition, appetitive single-cue trials were repeated at the most once due to the need to balance the numbers of appetitive and aversive single-cue trials. These features of the task allowed monkeys to predict that (1) an appetitive trial would follow an aversive trial (certainty of an appetitive trial), (2) an appetitive or an aversive trial would follow an appetitive trial that was preceded by an aversive trial (uncertainty), and (3) an aversive trial would follow 2 consecutive appetitive trials (certainty of an aversive trial).

To examine the impact of uncertainty and of aversive certainty relative to appetitive certainty on VP neurons, we compared their pre-CS period activity between situations (1 and 2) and between situations (1 and 3) (two-tailed *t*-test, $P < 0.01$, Bonferroni-corrected). This analysis revealed that 27 neurons (17%) out of 162 VP neurons exhibited a significant activity difference in the pre-CS period for these comparisons. Out of the 27 neurons, 11 (41%) showed a significant difference when comparing situations (1) appetitive certainly and (2) uncertainly. Moreover, 7 (26%) neurons showed differential activity in situations (1 and 3) aversive certainly, while 9 (33%) showed a significant difference in both comparisons (Fig. 7A). Thus, VP neurons appear to be modulated by uncertainty about the appetitive or aversive nature of the next trial as well as certainty about the aversive or appetitive nature of the next trial.

Finally, we classified the neurons into those preferring aversive situations in the future and those preferring appetitive situations. Out of the 27 neurons, 17 (63%) were negative context-preferring, that is, they showed higher activity in the pre-CS period when the next trial possibly or certainly was aversive (Fig. 7A; single example of neuron in Fig. 7B). The remaining 10 (37%) neurons were positive context-preferring, that is, they showed higher pre-CS activity when the next trial certainly was appetitive (an example neuron in Fig. 7C). Thus, a subpopulation of the VP was modulated by uncertainty and aversive certainty, which could underlie the generation of premature responses during the pre-CS period and the consecutive

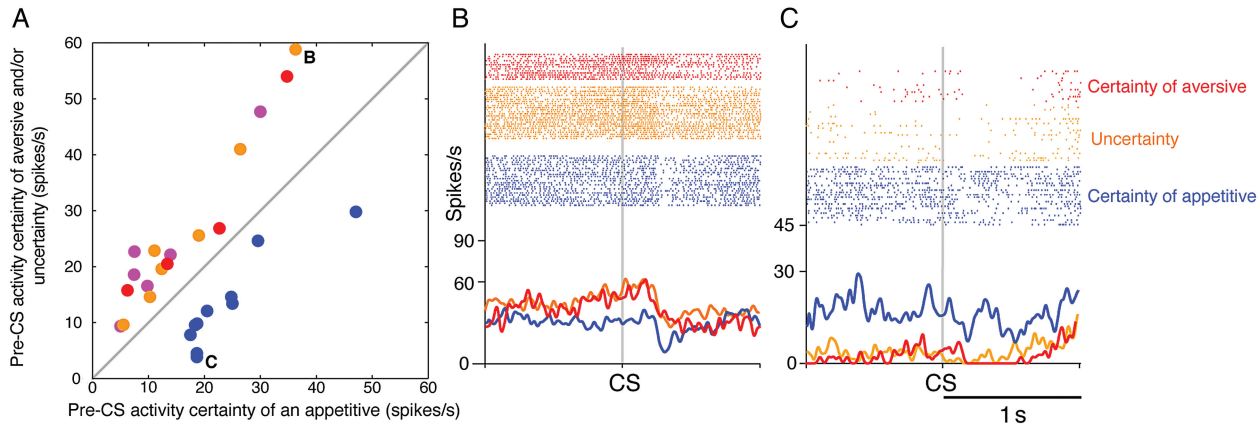

Figure 7. Pre-CS period activity modulated by possibility or certainty of next trial being aversive. (*A*) Pre-CS activity plot of individual neurons. The horizontal axis depicts the pre-CS activity in the situation for certainty of appetitive trial. The vertical axis depicts the pre-CS activity when the next trial was certainly or possibly aversive. Blue dots represent neurons preferring appetitive certainty ($n = 10$), orange dots represent neurons preferring uncertainty ($n = 7$), and red dots represent neurons preferring aversive certainty ($n = 4$). Magenta dots ($n = 6$) represent neurons activated by both aversive certainty and uncertainty. For both preferring neurons, the activities are represented by average activity in both situations. (*B*) Example of VP neuron corresponding to *B* in (*A*) that was preferentially activated both with uncertainty and with certainty of the next trial being aversive. Red, orange, and blue rasters and histograms represent activity when the next trial was certainly aversive, uncertain, and certainly appetitive, respectively. (*C*) Example of a neuron corresponding to *C* in (*A*) showing preferential activity for appetitive certainty.

premature responses after bicuculline injections. Without bicuculline, such an activity could in principle reflect the appropriate level of aversive anticipation and thereby prevent aberrant avoidance.

Discussion

Our mixed aversive and appetitive delayed response tasks in combination with bicuculline injections revealed that the VP plays a role in adaptive behavior. Bicuculline increased escape behavior (premature responses and omissions), particularly in aversive single-cue trials. Moreover, the VP neurons showed substantial responses in the aversive context, in addition to previously described responses in the appetitive context. The responses rarely occurred to both types of motivational events, suggesting that there are distinct negative context-preferring and positive context-preferring populations of VP neurons. It is conceivable that the negative context-preferring neurons underpinned the behavioral changes induced by bicuculline injections.

Different Behavioral Responses Under Different Motivational Contexts

In our delayed response tasks, the monkeys chose 1 of the 2 options in each trial. In the appetitive single-cue and dual-cue trials, they almost exclusively approached the CS associated with the appetitive US. In contrast, in the aversive single-cue trials, they mostly avoided the CS (>60% of complete trials). The fact that the performance in aversive avoidance was lower than appetitive approach behavior can be explained in 2 ways. First, a reaching movement (approach) is not the natural movement for avoidance. The avoidance response is more naturally linked to omission or nogo responses (Guitart-Masip et al. 2012), which is in-line with the greater difficulty to learn to avoid aversive outcome by an approach movement and the higher prevalence of omission errors in the aversive context. Second, in the aversive single-cue task, successful avoidance resulted in no aversive outcome, whereas only extinction of avoidance learning reproduces the primary reinforcer. Thus, repeated experience of the outcome may be necessary to consolidate and maintain intermediate levels of avoidance. In agreement with this view, a recent study in humans showed that feedback information about unchosen alternatives improves the avoidance learning (Palminteri et al. 2015) and thereby suggests an explanation for the relatively lower avoidance performance of our animals in situations without feedback in successful avoidance trials. Although the proportion of avoidance was variable, the best avoidance ratio was much higher than chance (see Behavioral Results), suggesting that the air-puff was indeed aversive and aversive CSs induced active avoidance behavior. Moreover, the monkeys showed more anticipatory blinking and incomplete trials but less anticipatory licking in aversive single-cue trials than in the other types of trials, indicating that they acquired different outcome anticipation (Fig. 2). These behavioral results are consistent with previous studies investigating aversive conditioning in monkeys (Paton et al. 2006; Belova et al. 2007; Joshua et al. 2009; Matsumoto and Hikosaka 2009a, 2009b; Morrison and Salzman 2011). Thus, CSs in the current study succeeded to provide different motivational contexts. Importantly, however, the CS-related and US-anticipatory activity of VP were typically opposite in aversive single-cue trials and appetitive single-cue trials. For example, if neurons were activated by an appetitive CS, they were typically inhibited by an aversive CS (Fig. 5A). This response pattern reinforces the behavioral finding that air-puff was indeed aversive and excludes alternative explanations, such as generalization (Mirenowicz and Schultz 1996) or higher-order conditioning (Tobler et al. 2003) of appetitive qualities to aversive trial events. However, from a strict conceptual point of view, the design of our task (without neutral CS) does not allow us to exclude that negative context-preferring CS activity is not an activity that predicts an aversive outcome but rather the reward omission. In any case, the motivational significance of positive and negative events was processed with opposing activities by VP neurons, indicating that the VP may play a role in the translation of opposed motivations into appropriate behaviors (approach or avoidance).

VP Dysfunction Increases Aberrant Escape Behavior in Aversive Contexts

The effects of bicuculline injections were relatively specific for aversive single-cue trials. This is in-line with the findings that

subject with Huntington's disease showing basal ganglia atrophy exhibit worse punishment-based learning (Palminteri et al. 2012). Moreover, the specificity of the effects suggests that general deficits in visual attention or motor control due to bicuculline are unlikely because the monkeys could still discriminate the CSs and execute actions to obtain rewards. Our tasks allowed monkeys to escape from performing trials without punishment. However, we required them to perform aversive trial in the single-cue task to perform appetitive trial, which was presented after aversive one. Thus, increased escape behavior induced by bicuculline suggests that the VP plays a role in the negative motivational state and control of their associated behaviors (escape or active avoidance) in different mechanisms (Seymour et al. 2015).

Another interesting finding in this study was the increase of heart rate by bicuculline injections. The heart rate increase under stressful experimental conditions would reflect a negative emotional state-like worry (Fisher and Newman 2013). Moreover, the heart rate could be changed by abnormal perceptual state in patients with neuropsychiatry diseases (Thayer et al. 2012). This finding is in line with this notion and so is the shared temporal profile of increased heart rate and increased premature responses. This suggests that disinhibition of the limbic territory of the indirect pathway or unbalanced activity between the direct and indirect pathways may change the internal physiological state and the behavioral expressions it engenders. These findings might explain why bicuculline injections into the VP enhance spontaneous stress-related behavior such as compulsive grooming and finger biting (Grabli et al. 2004).

The aversive anticipatory activity and bicuculline-induced premature responses observed in the monkeys in the present study may share some characteristics with clinical anxiety in humans. Patients with anxious-related disorders show hypervigilance, which involves excessive fear reactions to aversive stimuli as well as excessive aversive anticipation and excessive avoidance behaviors (Mogg and Bradley 1998; Reinecke et al. 2001; Mathews and MacLeod 2005; Nitschke et al. 2006; Bar-Haim et al. 2007). In our study, hypervigilance may explain the premature responses observed during the CS presentation period in the aversive single-cue and dual-cue trials. Importantly, premature responses in appetitive trials and dual-cue trails were unlikely to reflect impulsivity. We injected bicuculline unilaterally, resulting in enhanced attention toward the contralateral side in aversive single-cue trials. In the dual-cue task, the aversive cue was presented in proximity to the appetitive cue, which could lead to excessive reactions even in this context. The monkeys could still discriminate the CSs, execute actions to obtain rewards, and reach targets in appetitive single-cue trials. Thus, unilateral injection of bicuculline modulated their attentional state based on negative motivation.

Premature responses occurred also in the pre-CS period, even before the monkeys saw the CS on the screen. Excessive anticipation of aversive events could provide an explanation, particularly when the monkeys were uncertain whether the next trial would be aversive. This interpretation is supported by increased consecutive premature responses in the pre-CS period (Fig. 4*G*) during bicuculline injections and by anticipatory activities of VP neurons during the pre-CS period, particularly when the next trial could be aversive (Fig. 7). The findings may reinforce the intimate connection between excessive negative anticipation and aversive uncertainty, which is a major characteristic of anxiety disorders (Grupe and Nitschke 2013).

The fact that omissions were not increased in appetitive single-cue trials and in the dual-cue task argues against a general loss of motivation. Such apathetic states have been observed upon bicuculline injections into the lateral part of the VS in monkeys (Worbe et al. 2009) and in patients with lesions of the pallidum (Laplane et al. 1984, 1989; Levy and Dubois 2006). Instead, the omission behavior in aversive single-cue trials appears to be similar to the freezing reactions, which were observed after amygdala perturbations in rodents (Maren 2001) and during a threat or fear of aversive outcomes (Choi et al. 2010; Lazaro-Munoz et al. 2011; Bravo-Rivera et al. 2014). The fact that we found fewer omissions than premature responses may suggest that omission responses were for the monkeys to escape from performing the task due to being unable to overcome the aversive context through aversive CS. Taken together, bicuculline may have left the animals in a state of hypersensitivity and abnormal anticipation of potential aversive events, by which they tried to escape through premature responses or omissions.

VP Encodes Aversive Information

Our extracellular recordings confirmed previous findings of appetitive information encoding by VP neurons (Tachibana and Hikosaka 2012). We also found similarly extensive encoding of aversive contextual information, which had been considerably less well documented by previous studies (Calder et al. 2007; Joshua et al. 2009). Indeed, the proportion of neurons encoding aversive information in our study was more substantial (>38% of CS-related and 52% of anticipatory neurons), presumably because these aversive CS responses and the aversive outcome anticipation are expressed primarily in the context with only avoiding the aversive outcome. As shown in our results, a context of choices with appetitive alternative (Tachibana and Hikosaka 2012) does not promote the expression of neurons involved in aversive events or in encoding their avoidance. It is also easy to understand the difference with a Pavlovian task (Joshua et al. 2009), which does not need to activate these neurons to try to actively avoid aversive outcomes in a passive context.

The fact that appetitive information and aversive information were encoded by 2 different populations of VP neurons suggests that the VP neurons predominantly encode motivational contexts rather than the salience of stimuli. Accordingly, there were only few neurons showing similar activation by both appetitive and aversive information. This finding contrasts with the salience signals found in other brain regions, such as the ventral parietal cortex and the cingulate cortex (Kahnt and Tobler 2013; Kahnt et al. 2014). This finding corroborates the notion that the VP energizes motivated behavior with value information (Mogenson et al. 1980; Pessiglione et al. 2007; Tachibana and Hikosaka 2012). The VP neurons showed little activity specific for approach or avoidance behavior. Such information may be encoded more strongly by posterior and central parts of the GPe, where neurons projecting to motor cortical areas show action-selective responses (Yoshida and Tanaka 2009, 2015; Saga et al. 2011, 2013).

Similar to the observation with monkeys in the present study, the activation of the indirect pathway in rodents showed aversive behaviors (Hikida et al. 2010; Kravitz et al. 2010, 2012). The rodent studies thus support the notion that the direct and indirect pathways exert opposite effects on motivated behaviors.

We also found positive context-preferring neurons, raising several possibilities of functions of these neurons in the indirect pathway. First, to maintain appropriate motivational behavior, 2 populations are important to balance by interacting with each other. Second, positive context-preferring neurons were strongly suppressed in aversive single-cue trials (Fig. 6A), exerting also,

probably by striatal inhibition, a role in the aversive context, by blocking the approach and allowing active avoidance. Moreover, a direct interaction inside the VP between the 2 populations (positive context- and negative context-preferring) is also possible, given that VP neurons have axon collaterals that may affect the activity of their neighboring neurons (Smith et al. 1994; Matsumura et al. 1995; Shink and Smith 1995; Sadek et al. 2007). On the other hand, identification of the projection targets of these neurons would be important, but the VP may primarily play a role in controlling the negative context. Our findings from nonhuman primates go further by revealing the aversive context-related activity of the VP to overcome negative context to execute active avoidance. Its disturbance proves the causal role of the VP in the expression of escape behaviors that are frequently observed in anxiety disorders (Millan 2003). These findings prompt us to include this small structure of the indirect basal ganglia pathway in the map of regions that encode aversive information for negatively motivated behaviors.

Conclusions

We have shown that the VP plays a crucial role in encoding aversive contextual information and in controlling negative motivation to execute avoidance behavior in response to aversive cues and anticipation of consequence. The VP appears to be a player for translating motivation into appropriate action in aversive contexts. By extension, our data suggest that the VP could be an interesting target for studies on psychiatric disorders, such as OCD and PTSD.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes

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